CurveAlign V1.3 Beta2 User's Manual

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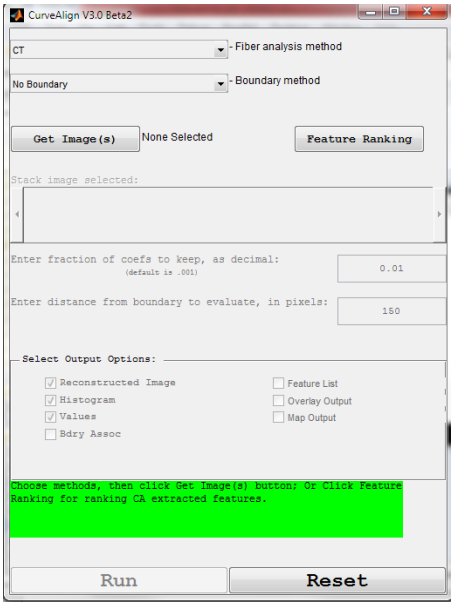
1 Introduction

The purpose of CurveAlign is to compute features that describe collagen interactions with epithelial cells. It was developed in order to search for stromal changes that are correlated with disease in images of collagen and epithelial cells. We have recently used CurveAlign for the feature calculations performed in a research paper by Bredfeldt, et. al. (Journal of Pathology Informatics, 2014, in press). Collagen images may be obtained by a number of imaging approaches however we have focused here on Second Harmonic Generation (SHG) images of collagen. Epithelial cell information is input into CurveAlign as an 8-bit mask file, that must be pre-registered with the collagen image, where white pixels correspond to epithelial cell regions and black pixels correspond to anything else in the image i.e. the background, collagen fibers etc. These mask files can be generated by any appropriate means, such as manual ROI annotation in FIJI or using segmentation tools in MATLAB or FIJI. The output features can then be used to potentially classify images or fibers using machine learning techniques. An Support Vector Machine (SVM) is incorporated into CurveAlign for ranking extracted features, and these features can also be used or ranked through many open source machine learning tools such as Weka and R.

The primary change in CurveAlign Beta version 3.0 is feature extraction from CT-FIRE output. Compared to the version 2.3, the major updates are : (1) output up to thirty four fiber features, including angle, alignment, density, and etc. , these features are saved in both .cvs file and .mat file, which can then be used to potentially classify images or fibers using machine learning techniques; (2) tiff boundary can be loaded to investigate the interaction between fiber and cell boundary; (3) CT-FIRE fiber extraction results can be read in; (4) a feature ranking using support vector machine (SVM) is included; and (5) keep the most features of the older version (2.3), such as the statistics for the relative angles, heat map to show the fiber(angle) alignment, and the fiber-boundary association, etc.

The GUI in CurveAlign is modular, so that the main user interface is in a separate window from the outputs. This allows for the user to resize the output windows to their preferred size. The main user interface window is shown below.



In this manual, depending on the selected "Fiber analysis method" in the drop down box at the top of the GUI, "fiber" may refer to curvelets that represent a fiber segment (CT), fiber segments generated by CT-FIRE, the extracted fiber generated by CT-FIRE , or extracted fiber endpoints generated by CT-FIRE.

2 QuickStart Instructions

1) Select the fiber processing mode from among the following options- CT,CT Fire Segments, CT FIRE endpoints and CT FIRE Fibers
2) Select the boundary processing mode from among the following options- No Boundary, CSV Boundary , Draw Boundary and TIFF Boundary.
3) Click "Get Images" and browse to an image. Images may be single or stacks. If you would like to process more than one image in a batch, just select more than one image in the dialog using the technique appropriate for your operating system (i.e. in Windows, hold CTRL while selecting multiple files).
4) If a manual boundary is required (not available in batch mode), hold down the Alt key and click points along the desired path on the image window. Release the Alt key when finished.
5) Enter the keep threshold level if fiber extraction mode "CT" is selected, and select the desired outputs using the checkboxes on the bottom of the main window.
6) Click the run button.
7) The output data and images will automatically be placed in a folder called CA_Out in the directory with the original image(s).
8) If you have drawn a boundary, you will be prompted to give a file name and location for the boundary points to be saved. These will be saved as a *.csv file. This file can be used again in the future by selecting CSV boundary as the boundary processing mode. Store the boundary in the same directory as the original image with the filename as "image.tif.csv" if the name of the original image is" image.tif".
9) The current function being performed by CurveAlign is listed in the Status label at the bottom of the main window.

3 Relationship with CT-FIRE

CT-FIRE is another software tool developed for processing images of fibers (Bredfeldt J., et al., Journal of Biomedical Optics, 2014). CT-FIRE traces fibers and outputs a database of fiber objects. This output database can be used as an input to CurveAlign (the subject of this manual). CurveAlign's main purpose is to compare fibers with boundaries and fibers to each other to measure density and alignment. When CT-FIRE inputs are used, the fibers are compared to region boundaries, either drawn manually, or created by any segmentation tool.

CT-FIRE and CurveAlign may someday be combined, but the basic philosophy is that it is useful to have them separate and easy enough to just connect them by reading the CT-FIRE outputs into CurveAlign when needed.

4 Fiber processing modes

There are four fiber processing modes. To avoid errors, ensure that the previously opened xls files of results of an image are closed before any other operation is performed on the same image

1) Curvelet Transform: The curvelet transform is performed on the image(s) and each curvelet corresponds to an observation in the feature list. Each curvelet has a unique angle and position. In this case, fiber length and curvature are not available, since curvelets are independent from one another.
2) CT-FIRE Segments: The output from CT-FIRE is used as the input, providing information about the fibers in the image. Each fiber segment in the CT-FIRE output file corresponds to an observation in the feature set. Each segment has a unique angle and position. In this case, each segment is given a fiber length and curvature value that corresponds to the length and curvature of the entire fiber this segment belongs to. For this option, the .mat file generated by CT-FIRE must be saved in the same folder as the original .tif image, and when prompted the.tif image should be selected.

3) CT-FIRE Fibers: The output from CT-FIRE is used as the input, providing information about the fibers in the image. Each fiber center point in the CT-FIRE output file corresponds to an observation in the feature set. Each center point has a unique angle corresponding to the average angle of the fiber. The position is the midpoint between the fiber end points. In this case, each center point is given a fiber length and curvature value that corresponds to the length and curvature of the entire fiber this center point belongs to. For this option the .mat file generated by CT FIRE must be in the same folder as the original .tif image, and when prompted the.tif image should be selected.

4) CT-FIRE Fiber Endpoints: The output from CT-FIRE is used as the input, providing information about the fibers in the image. Each fiber endpoint in the CT-FIRE output file corresponds to an observation in the feature set. Each endpoint has a unique angle and position. The angle corresponds to the angle of the entire fiber. In this case, each endpoint is given a fiber length and curvature value that corresponds to the length and curvature of the entire fiber this segment belongs to.

When CT-FIRE outputs are used as inputs to CurveAlign, the CT-FIRE output files must be named according to a strict naming convention. For example, if the image is named the following:
1B-a1.tif
Then the CT-FIRE output file must be named the following:**ctFIREout_1B-a1.mat**

At the time of writing this manual, the above naming convention has been adopted by the CT-FIRE tool as well, so the outputs from CT-FIRE may be directly used as inputs to CurveAlign.

5 Boundary processing modes

There are four boundary processing modes.

1) No boundaries: Fibers will be compared to each other, but not to a boundary. For example, features about fiber alignment will still be computed and output in the feature list.

2) Manual boundaries: The user is required to manually draw boundaries with the Alt-key + mouse clicking technique. This option is not allowed if multiple input images are selected to be processed in a batch. If this option is selected and multiple files are to be processed, then the "No Boundaries" option is automatically selected. All boundary points must be selected in a succession, to select a new boundary click anywhere on the image and then select a new boundary.
3) CSV boundaries: Previously stored boundary files can be used that contain the x-y positions of points along the boundary.
    a. CSV files must adhere to the following naming convention. If the image is named: TACS-3a.jpg, then the CSV file must be named: boundary for TACS-3a.jpg.csv.
    b. The boundary file must be in the same directory as the original image.
4) Tiff boundaries: Mask files are used to indicate where region boundaries are located. The mask files should be 8-bit files where the inside of ROIs are given a value of 255 and outside a value of 0. This allows for features identifying whether a fiber is inside an ROI or not. These files must be in pixel for pixel registry with the image files and therefore have the same dimensions as the image files. Tiff boundary files must adhere to the following naming convention. If the image is named: 1B-a5.tif, then the tiff boundary file must be named: mask for 1B-a5.tif.tif . The boundary file must be in the same folder as the original image

When boundaries are used as part of the analysis, CurveAlign computes up to 2 relative angles per observation. The first is the nearest point angle and the second is the intersection point angle. The nearest point angle is the relative angle between the observation point and the tangent angle of the region boundary at the point nearest to the observation point. The intersection point angle is the relative angle between the observation point and the intersection point of the fiber, interpolated out a user defined distance, and a boundary.

6 Input files

CurveAlign requires up to three types of input files.

Image files: These files contain images of fibers. These can be produced by any method available, however we have particularly focused on SHG images of collagen fibers.

CT-FIRE files: The output of CT-FIRE having been run on the above mentioned "Image files."

Boundary files: The boundary files can be CSV files or Tiff files. See the section about Boundary Modes above to learn about naming conventions for these file types.

.mat files must be present in the same directory as the original image while using the results of the CT FIRE results.

7 Output data files

There are several files that are generated by the CurveAlign software for each image processed. These files and their descriptions are listed in the table below:

Filename	Description
*_hist.csv	List of bin values and numbers of observations in each bin. Bin values correspond to relative angle in if boundaries are used, and absolute angle if no boundary is used.
*_values.csv	relative angles as in version 2.3(using the nearest distance the defined the region of interest)
*_stats.csv	Statistics of the relative angles as in version 2.3
*_fibFeatNames.csv	Names of the 34 features
*_fibFeatures.csv	34 features in 34 columns in the order in *_fibFeatNames.csv
*_fibFeatures.mat	Features that can be used for the feature ranking here
compFeat.mat	Feature ranking output: feature array and meta array
featRank.txt	Feature ranking output, including feature importance and normalized feature difference between two classes

8 Output images

A few output images are saved in .tiff format in the selected output directory. These images are explained below.

8.1 Overlay image

This image shows the position of each fiber detected within the particular distance from the user defined boundary. The fibers outside the specified distance are also plotted for

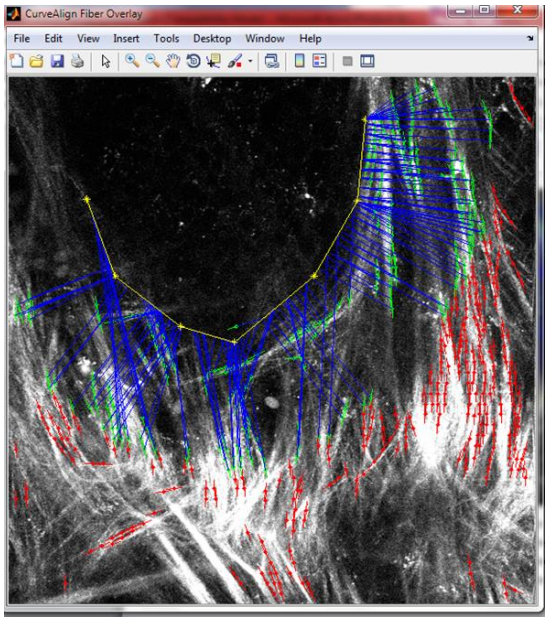


Figure 1. Overlay image showing the fibers that are within or outside the specified distance. The green lines show the fibers inside the specified distance. The red lines show the fibers outside the specified distance. The boundary is the line connecting the yellow mark "*". Blue lines are the lines connecting the centers of the fibers with the nearest points on the user defined boundary.

8.2 Map Image

The map image is intended to help the user identify the spatial distribution of fiber angles within the image.

The raw map (_rawmap.tiff) file codes the angle of the fiber into a grey scale value. The pixel in the image where the center of the fiber is located is given a value between 0 and 255 that corresponds to 0 to 90 degrees when a boundary is selected and 0 to 180 degrees when a boundary is not selected. This file may be further processed in matlab or imagej according to the users preferences.

The processed map file (_procmap.tiff) is a processed version of the raw map file overlaid on the original image. The output is intended to show regions of aligned structures that are perpendicular to the boundary, in the case of a boundary selection, or regions of generally aligned structures, in the case where no boundary is selected.

How processed map files are created:

When a boundary is selected, the raw map file translates 0 to 90 degrees into 0 to 255 in gray scale. The center location of each curvelet is given a gray level corresponding to its angle with respect to the boundary. Then a square max filter is applied with a size of 12 pixels on a side, followed by a Gaussian disc filter with a sigma of 4 pixels. The color scale is then set to 0-10 degrees = black, 10-45 degrees = green, 45-60 degrees = yellow, and 60-90 degrees = red. The map image is overlaid on the original image with transparency set to 0.5 as shown in **Error! Reference source not found.**.

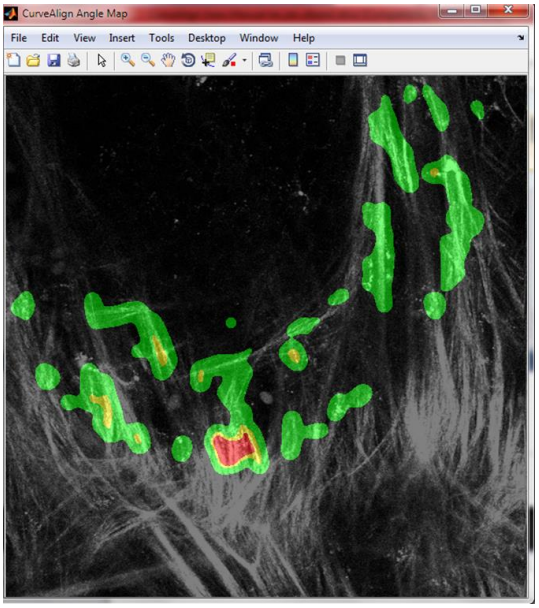


Figure 3. Map image of the overlay image showing the fibers that are within the specified distance.

When a boundary is not selected, the raw map file translates 0 to 180 degrees into 0 to 255 in gray scale. In this case, red indicates areas of highly aligned structures, while black and green indicate areas of more randomly aligned structures. The map image is overlaid on the original image with transparency set to 0.5 as shown in **Error! Reference source not found.**.

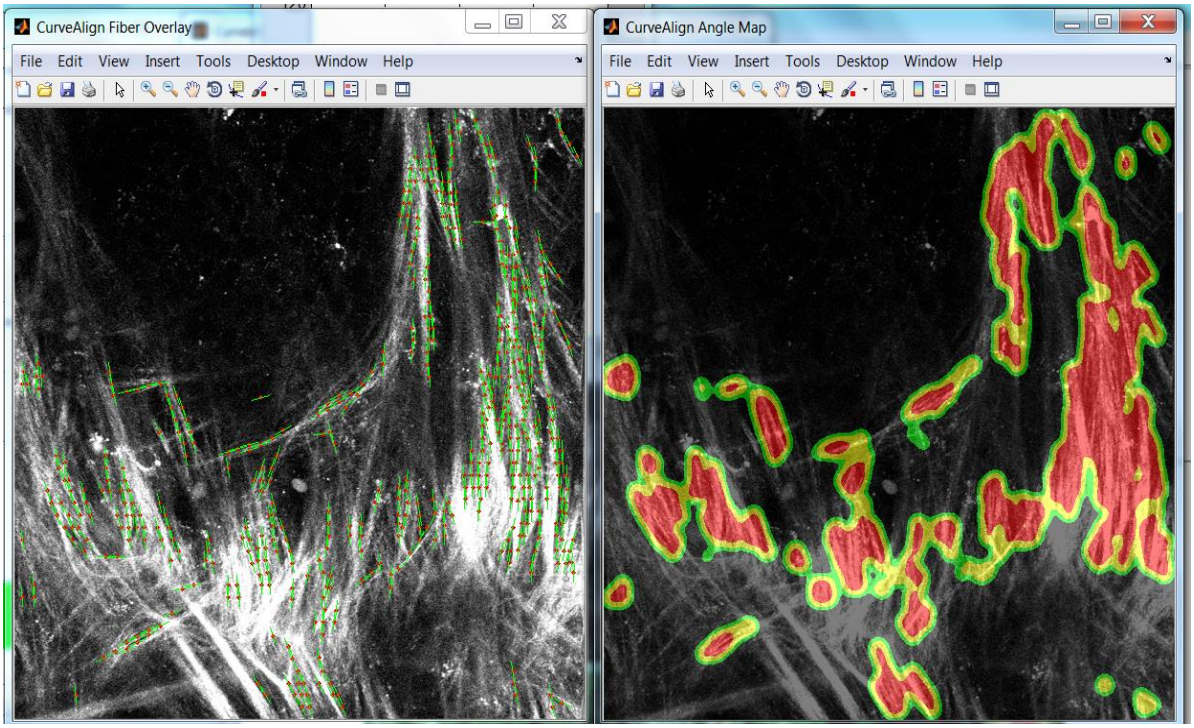
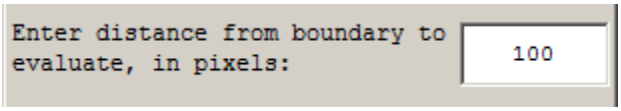

CurveAlign Fiber Overlay
File Edit View Insert Tools Desktop Window Help
CurveAlign Angle Map
File Edit View Insert Tools Desktop Window Help

Figure 2. Overlay and map images when no boundary is selected.

Boundary Analysis

The user is able to analyze the fibers that fall only within a certain distance from a boundary. To enter this distance, first a boundary must be drawn or opened from file. Then the distance in pixels should be entered into the edit box.



Boundaries are created by alt-clicking on the original image that is opened in the CurveAlign GUI. When alt is released, the boundary is ended and no additional points may be added to the boundary. If the boundary mode "CSV Boundary" or "Tiff Boundary " is selected, previously saved boundary file can be automatically checked and loaded in if it exists. The boundary file should be saved in the same directory as the selected image and should be named like the following:

Image file name = TACS-3a.jpg

Boundary file name = boundary for TACS-3a.jpg.csv

or

Boundary file name = mask for TACS-3a.jpg.tif

The position on the boundary that is being compared to each fiber may be visualized as well. This allows the user to see where the angle comparisons are being made. Blue lines are drawn on the overlay output image from each fiber to the point on the boundary that the fiber is associated with.

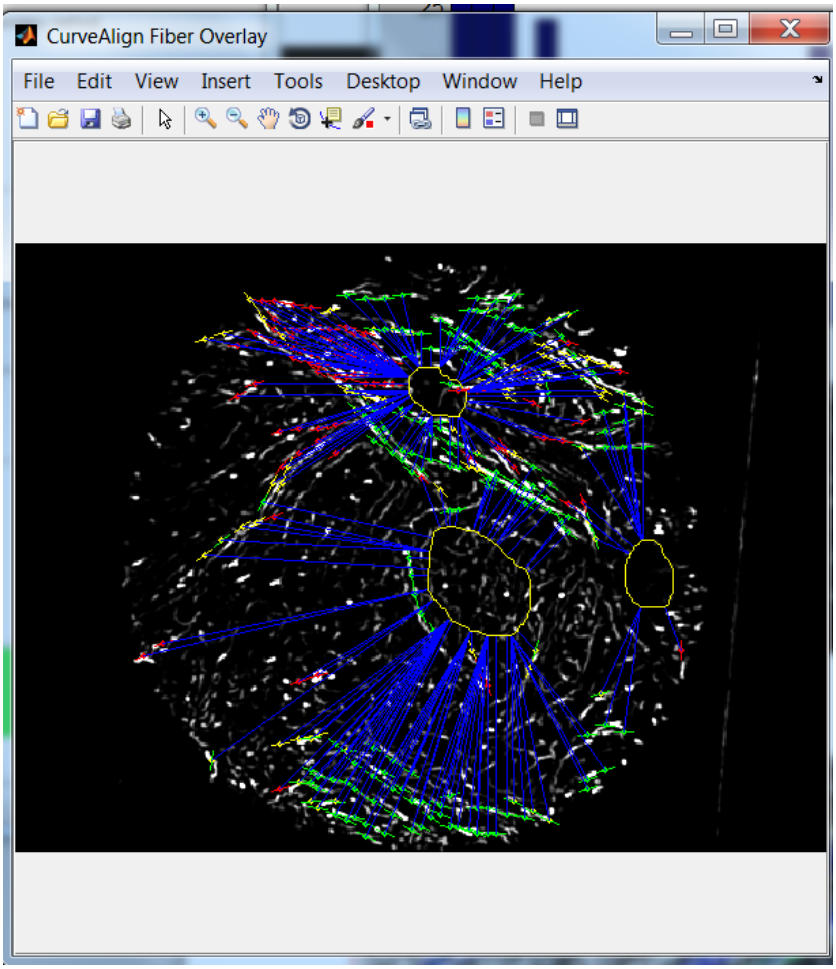
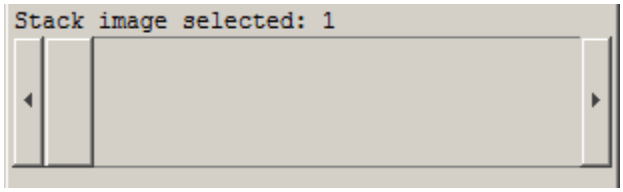


Figure 3. An example of an overlay images where the associations between fibers and the boundary are indicated by blue lines.

9 Stacks

CurveAlignV3.0 allows for the user to select stacks of images. When a stack is opened, the slider bar is enabled, allowing the user to choose which image in the stack should be displayed.



When a stack is being analyzed, then the output files that are images will also be in stack format. To open these stacks in ImageJ, the LOCI bioformats importer must be used. For some reason, these tiff stacks cannot be drag and dropped into ImageJ, this will be investigated further in the next release of CurveAlign.

The other output files, for example the histogram file and the curvelet angle spreadsheet, are produced such that each image in the stack creates a new output file. For example a stack with 4 images will generate 4 histogram files named stack_1_hist.csv, stack_2_hist.csv, stack_3_hist.csv, etc.

9.1 Stacks with Boundaries

If a stack is being processed, then only a single boundary can be used for the entire stack. Future versions of this tool will allow for a different boundary in each image plane in a stack.

9.2 Stacks without Boundaries

When no boundary file is selected, then each image in the stack is analyzed as described in the section above about image analysis without a boundary.

10 Batch mode

Batch mode is used to process multiple images and potentially associated boundaries. To use batch mode, click the Get Images button and select more than one image in the file selection dialog box. Each image will be processed according to the modes currently selected.

CurveAlign will first search for boundary files in the chosen directory. If there are boundary files, then it will process all images that are found to be associated with the located boundary files. Images may be a mixture of both individual images and stacks. If the image is a stack, then the entire stack will be processed.

The batch mode output files will be stored in a directory named CA_Out and will include all of the outputs available from the CurveAlign software.

10.1 Batch mode with CSV boundaries

If boundaries are drawn with the CurveAlign program, they should be saved in the same directory as the images. The boundary files should be named like the following:

Image file name = TACS-3a.jpg

Boundary file name = boundary for TACS-3a.jpg.csv

10.2 Batch mode without boundaries

To process a directory of images without boundaries, then just place the images in a directory by themselves (without any boundary files), then run CurveAlign with select all of the images in that directory. In this case, the distance from the boundary and boundary association edit boxes will be ignored.

10.3 Batch mode with Tiff boundaries

In batch mode, boundaries may also be imported as tiff files. Tiff boundary files must be 8bit binary mask images where the inside of a region has a value of 255 and everything else must be 0. Boundary tiff files must have the same number of pixels (length and width) as the original image and may be produced by hand or by segmentation in ImageJ/FIJI. Tiff boundary files should be named according to the following convention:

Image file name = 1B-a5.tif
Boundary file name = mask for 1B-a5.tif.tif

10.4 Batch mode with CT-FIRE input

To compare the results of the CT-FIRE software to boundaries (either TIFF or csv), then choose one of the CT-FIRE fiber processing modes on the main GUI. The following naming convention should be strictly used:

Image file name = 1B-a1.tif
CT-FIRE results file = ctFIREout_1B-a1.mat

11 Feature Ranking

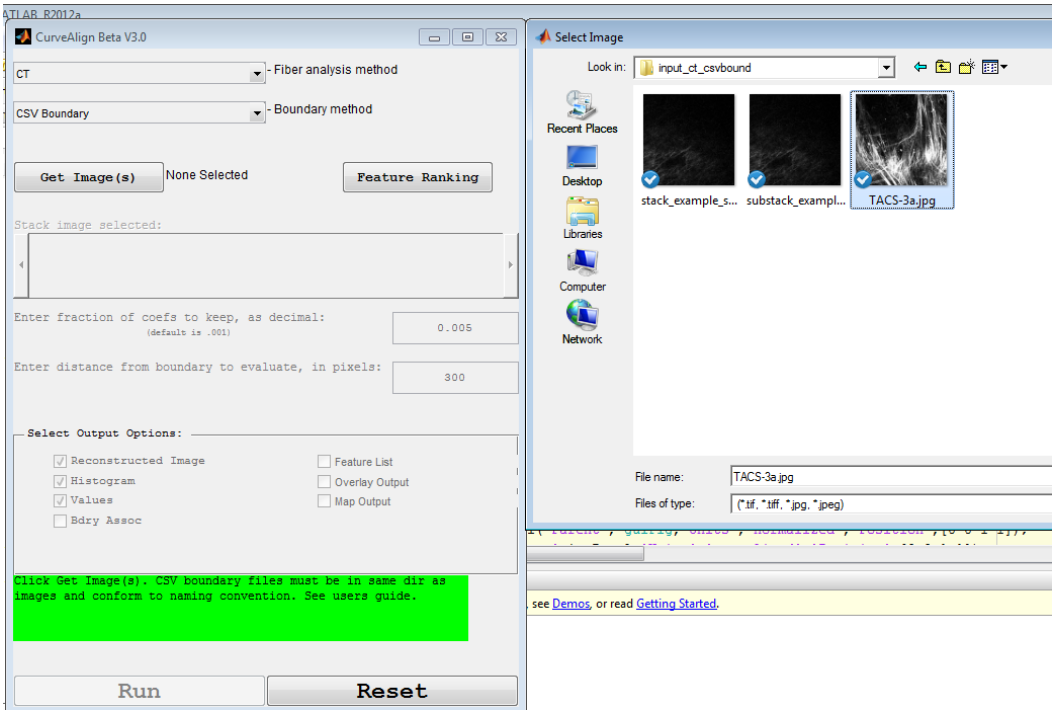
Based on the importance for differentiating two-class classification problem (Negative and Positive) using SVM, SVM feature weight is used to rank the features. The average feature value difference between two classes is also included. An annotation file including the label for each image is needed to be prepared before running the feature ranking. This file is named as "annotation.xlsx" with two columns, where the first column includes the label for each image(e.g.: 1 for positive, 0: negative), the second column includes the full original image name.

12 Tutorials

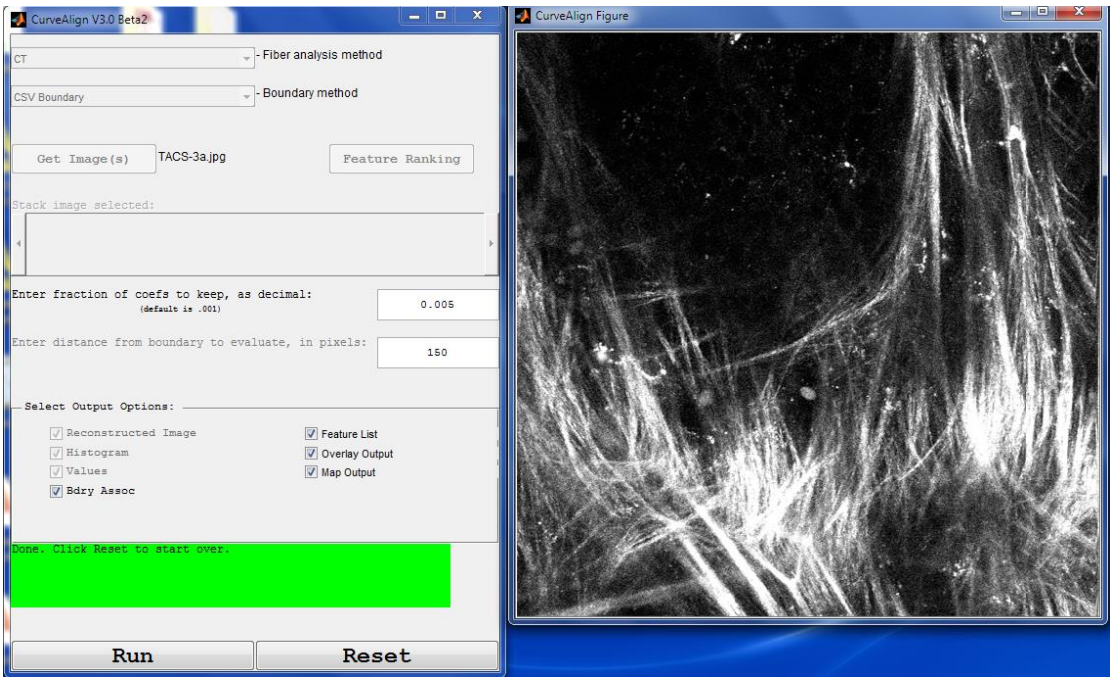
To reproduce the tutorials, users need to first download the test images and boundary files at LOCI website.

12.1 Tutorial 1: feature extraction with fiber extraction method "CT" and CSV boundary

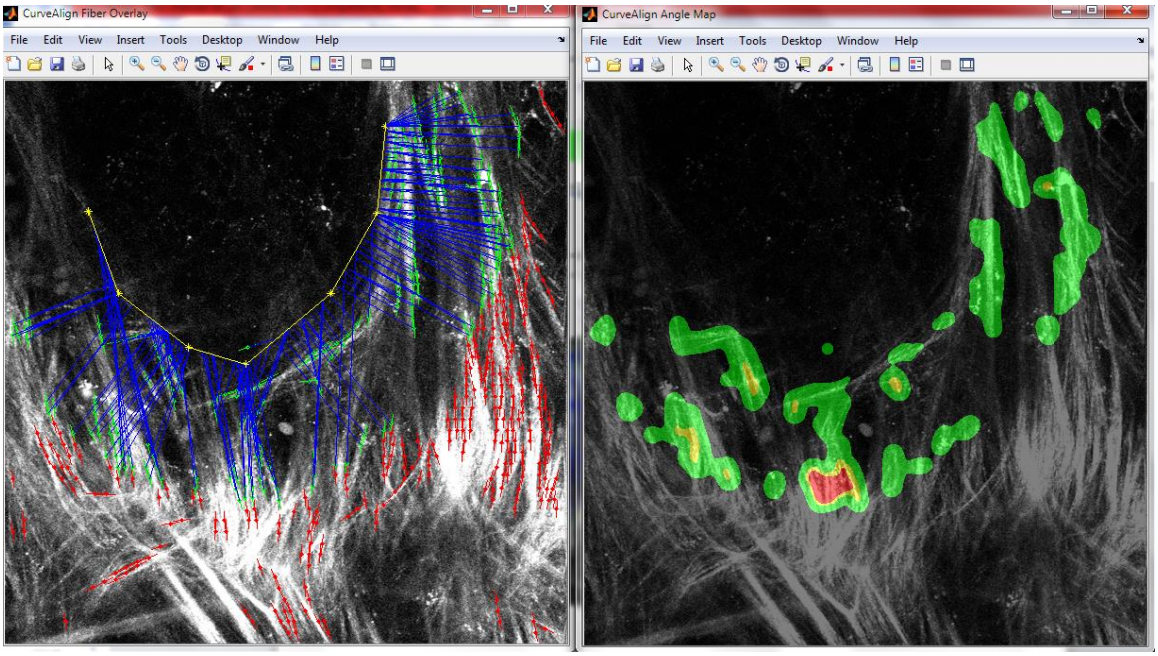
Step 1 choose the methods of fiber extraction and boundary, open an image: As shown in the Figure below, Select the "CT" as the Fiber analysis method, "CSV Boundary" as the Boundary method and then click the "Import image/data" button. A file selection window opens allowing the user to choose the image. In this tutorial, "TACS-3a.jpg" is selected.



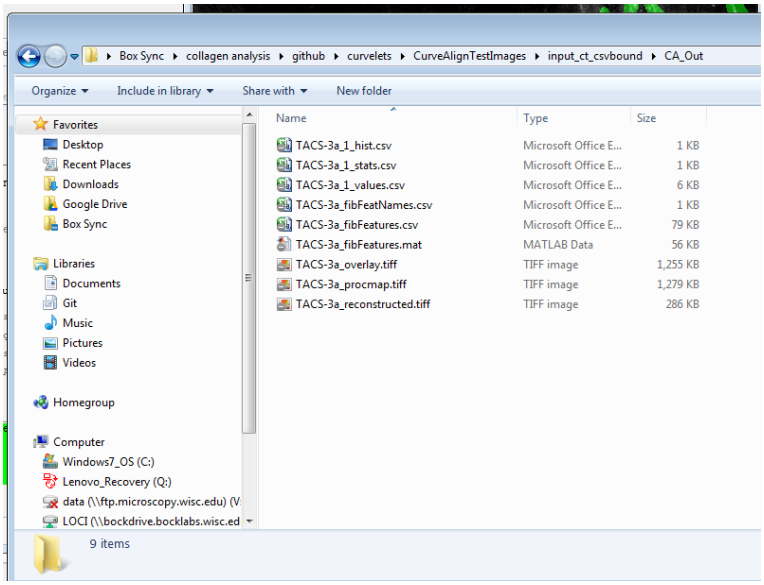
Step 2 select run parameters and set output options: After clicking "Get Images", the image is displayed and new controls are enabled on the control panel as shown below, and information shown in the command window "Found boundary for TACS-3a.jpg.csv" indicates the CSV boundary file was found. The fraction of curvelets coefficients is set to "0.005", the region of interest is set to the area within the 150 pixels distance from the boundary. All the output options are checked.



Step 3 run fiber feature extraction: click "Run" button. Then, the progress will be listed in the Command Window. The overlaid and map images will also be displayed as follows


CurveAlign Fiber Overlay
File Edit View Insert Tools Desktop Window Help
CurveAlign Angle Map
File Edit View Insert Tools Desktop Window Help

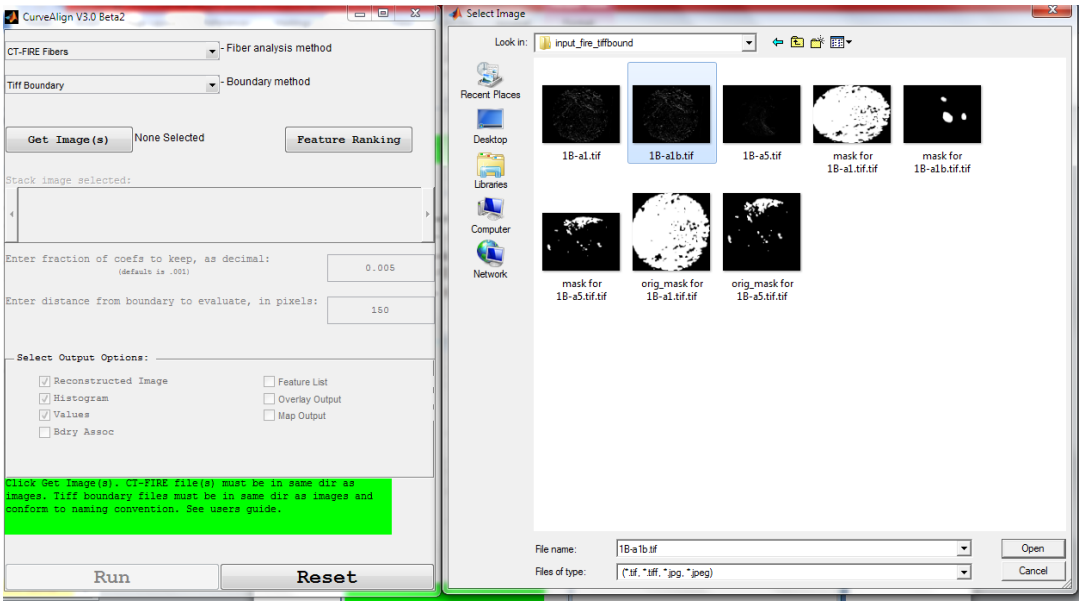
Step 4 check the results: All the results are automatically saved to a new subfolder called "CA_Out"within the folder where the image exists. In this case, the path for this subfolder is " C:\Users\youmap\Desktop\Box Sync\collagen analysis\github\curvelets\CurveAlignTestImages\input_ct_csvbound\CA_Out\)". Nine output files are shown:



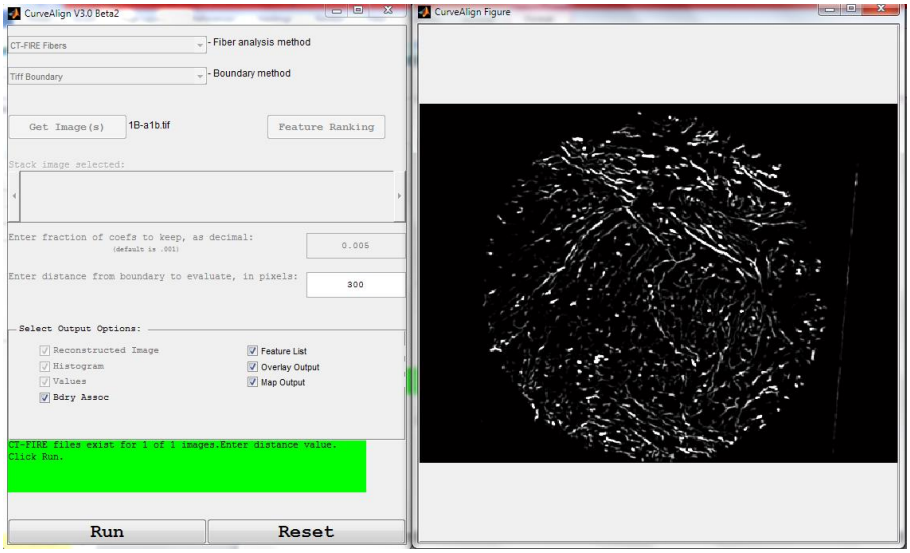
Step 5 reset the GUI: Click "Reset" to start over .

12.2 Tutorial 2: feature extraction with CT-FIRE method and Tiff boundary

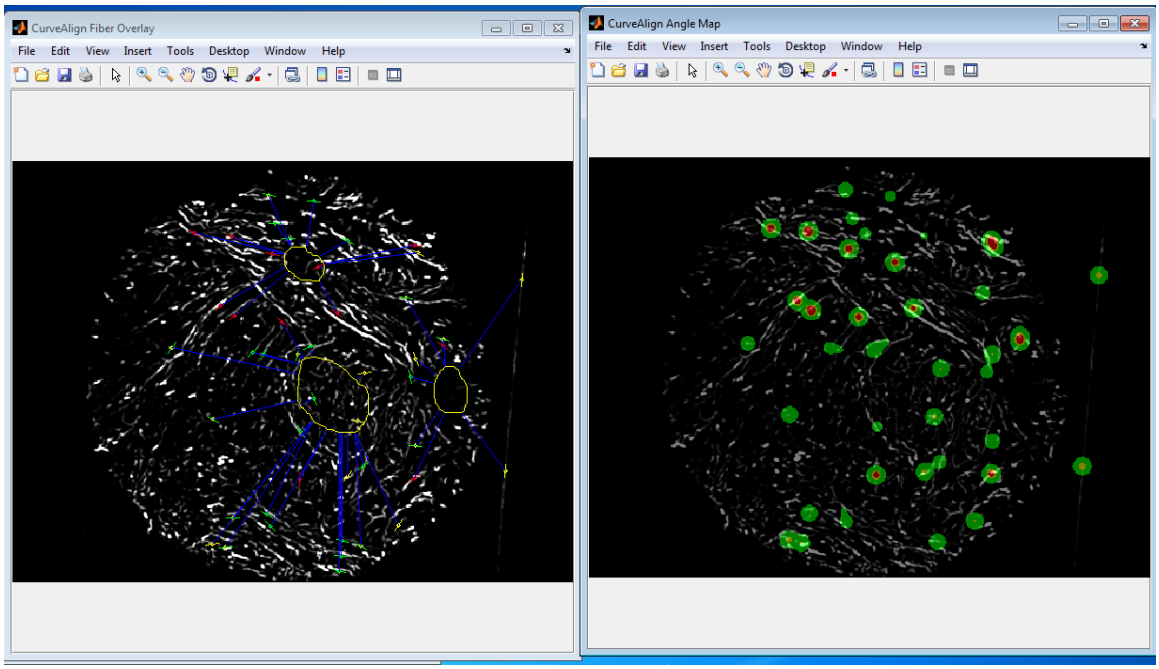
Step 1 choose the methods of fiber extraction and boundary, open an image: As shown in the Figure below, Select the "CT-FIRE Fibers" as the Fiber analysis method, "Tiff Boundary" as the Boundary method and then click the "Import image/data" button, a file selection window opens allowing the user to choose the image. In this tutorial, " 1B-a1b.tif" is selected.



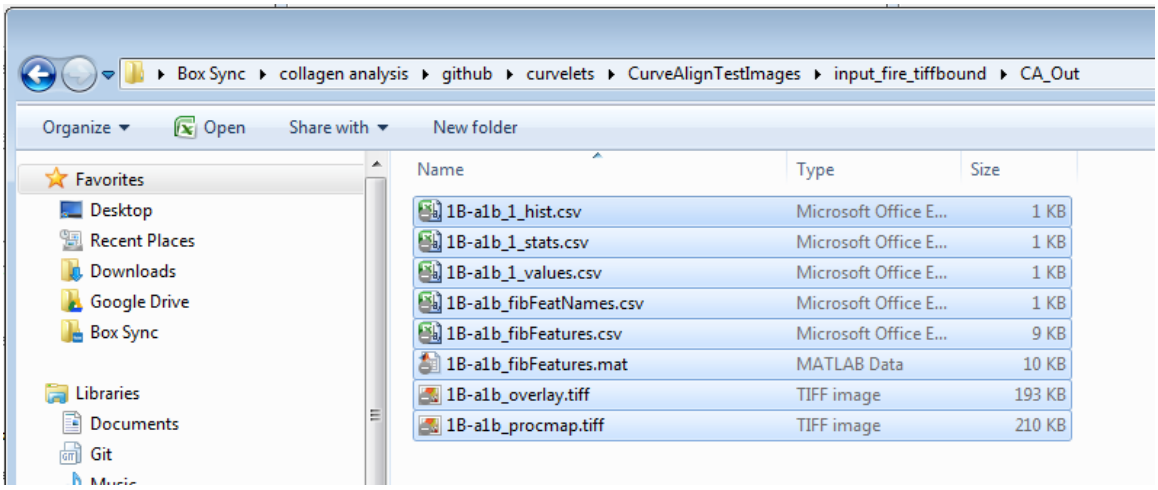
Step 2 select run parameters and set output options: After clicking "Get Images", the image is displayed and new controls are enabled on the control panel as shown below, and the information shown in the command window " Found ctFIREout_1B-a1b.mat, Found mask for 1B-a1b.tif.tif" indicates the CT-FIRE output file and Tiff boundary file were found. The region of interest is set to the area within the 300 pixels from the boundaries. All the output options are checked.



Step 3 run fiber feature extraction: click "Run" button. Then, the progress will be listed in the Command Window. The overlaid and map images will also be displayed as follows


CurveAlign Fiber Overlay
File Edit View Insert Tools Desktop Window Help
CurveAlign Angle Map
File Edit View Insert Tools Desktop Window Help

Step 4 check the results: All the results are automatically saved to a new subfolder called "CA_Out"within the folder where the image exists. In this case, the path for this subfolder is C:\Users\youmap\Desktop\Box Sync\collagen analysis\github\curvelets\CurveAlignTestImages\input_fire_tiffbound\CA_Out\)". Eight output files are shown. Compared to the output of the tutorial 1, the CT-reconstructed image is not available here.

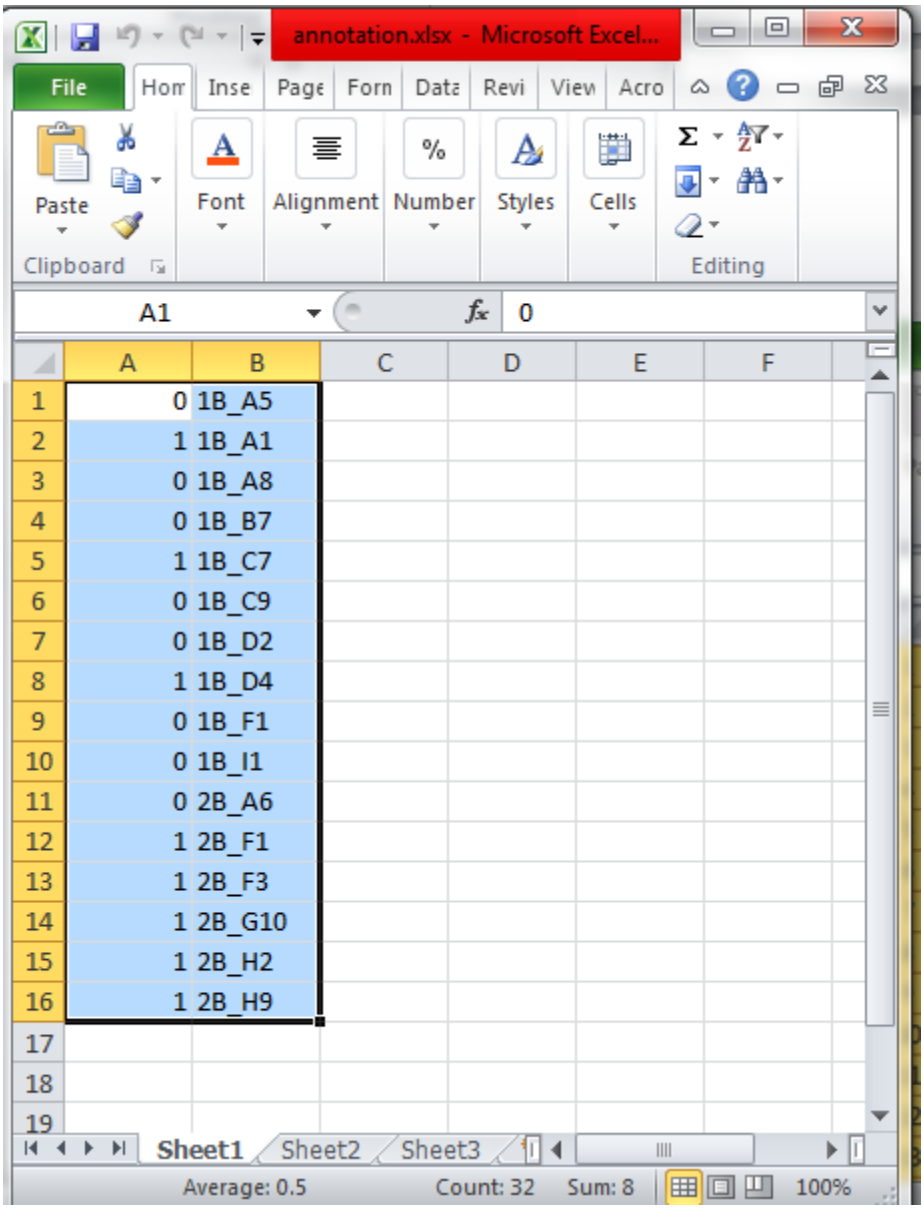


Step 5 reset the GUI: Click "Reset" to start over .

12.3 Tutorial 3: feature ranking

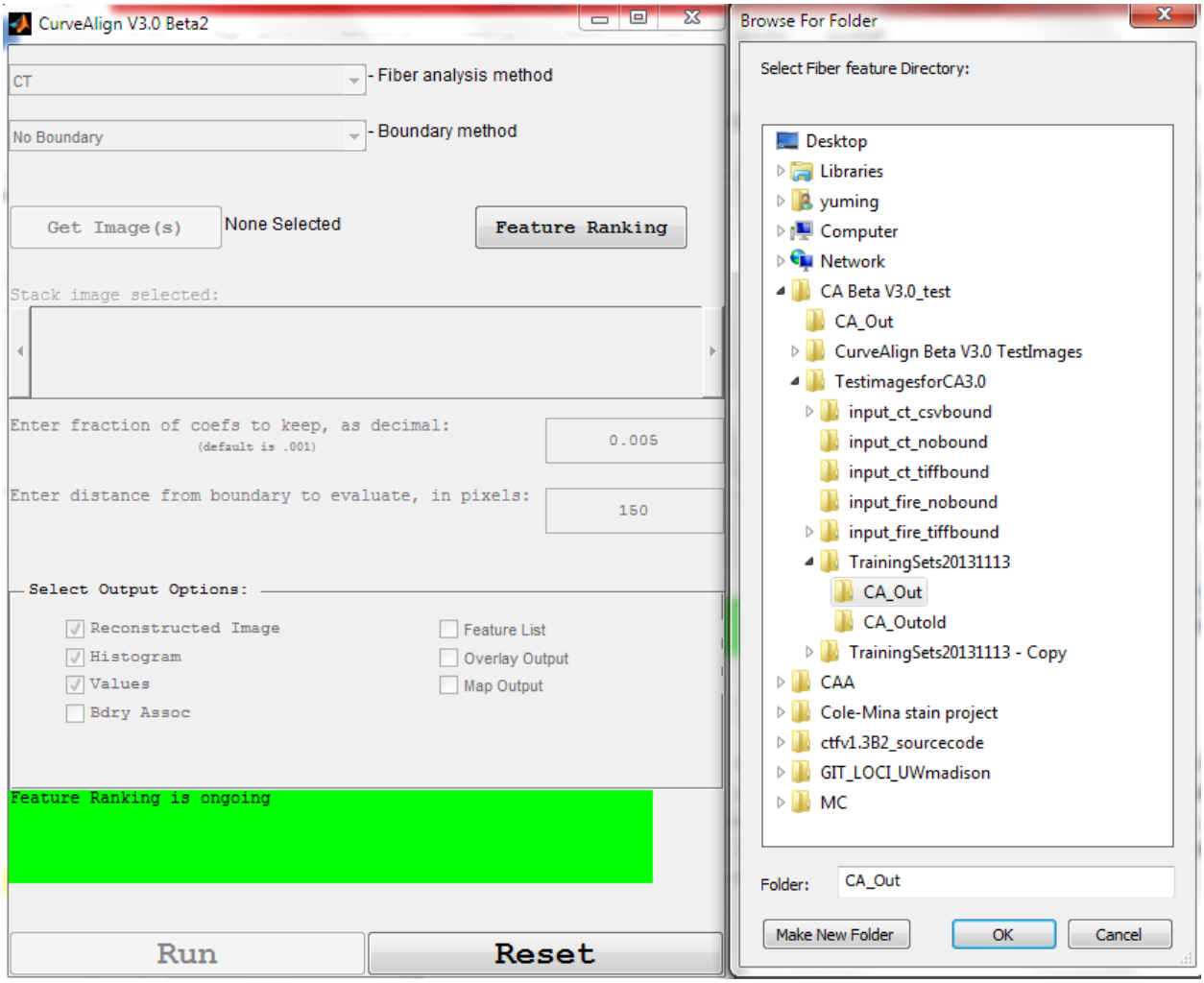
This tutorial shows how to rank features for a training data set that includes 8 TACS-3 negative images and 8 TACS-3 positive images.

Step 1 prepare annotation file: The file is named "annotation.xlsx" which contains the label for each image and must be saved in the same folder as the CA extracted feature files, i.e the foder of /CA_Out/. The annotation file for this training images is shown below,

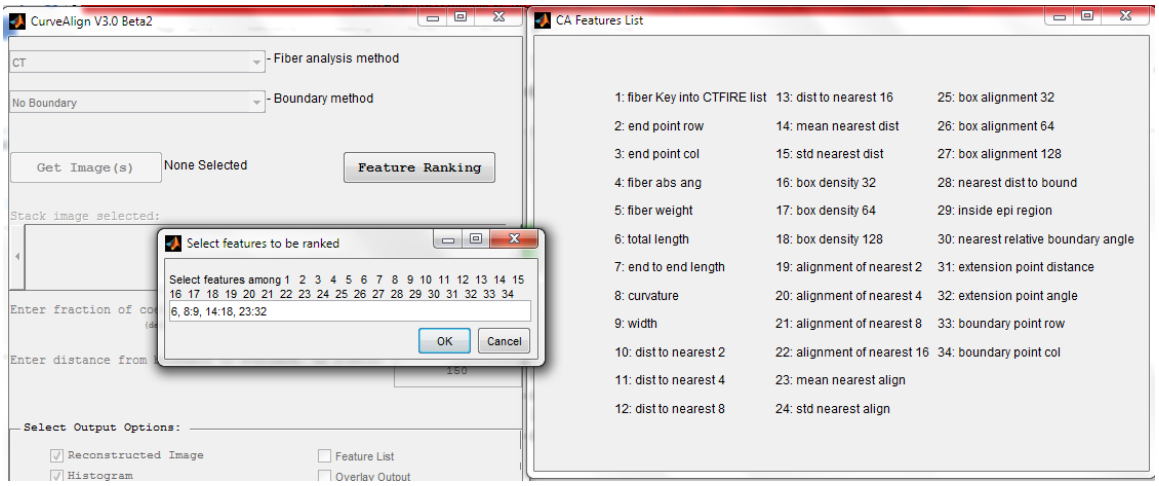


wherein, 0 indicates the image is TACS-3 negative, 1 indicates the image is TACS-3 positive

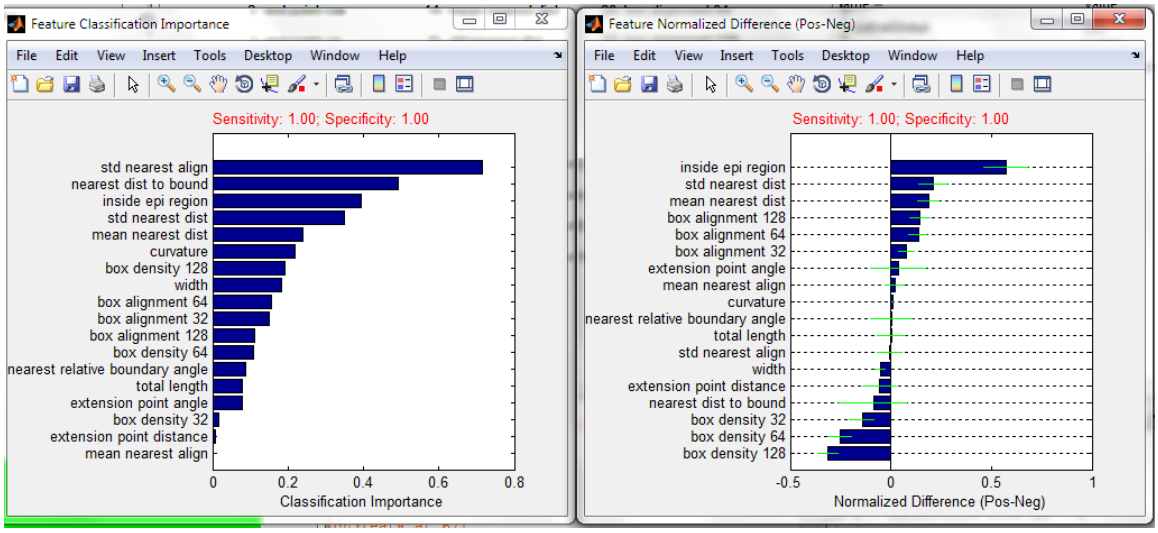
Step 2 Run feature ranking: click the Feature Ranking button, all other fiber feature extraction buttons are "enabled off" and a window is popped up to "Select fiber feature directory". Here, "\TrainingSets20131113\CA_Out\" is selected as shown below



After clicking OK, two windows pop up as shown below. One is the CA Features List showing the index and description of each of the 34 features , the other is a input dialog window which shows the applicable features and can be used to enter the number of the features to be ranked. In this tutorial, enter " 6, 8:9, 14:18, 23:32 " as shown below , i.e features [6 8 9 14 15 16 17 18 23 24 25 26 27 28 29 30 31 32]. User can input arbitrary number of features to be ranked.


CurveAlign V3.0 Beta2
Select features to be ranked
Select features among 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34
6, 8:9, 14:18, 23:32
OK
Cancel
CA Features List
1: fiber Key into CTFIRE list
2: end point row
3: end point col
4: fiber abs ang
5: fiber weight
6: total length
7: end to end length
8: curvature
9: width
10: dist to nearest 2
11: dist to nearest 4
12: dist to nearest 8
13: dist to nearest 16
14: mean nearest dist
15: std nearest dist
16: box density 32
17: box density 64
18: box density 128
19: alignment of nearest 2
20: alignment of nearest 4
21: alignment of nearest 8
22: alignment of nearest 16
23: mean nearest align
24: std nearest align
25: box alignment 32
26: box alignment 64
27: box alignment 128
28: nearest dist to bound
29: inside epi region
30: nearest relative boundary angle
31: extension point distance
32: extension point angle
33: boundary point row
34: boundary point col

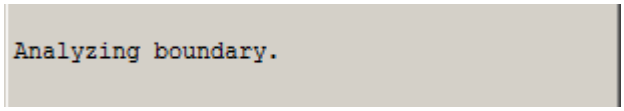
After clicking "OK" button on the feature selection window, feature ranking starts and the two output windows are displayed as below, including both the feature classification importance and feature normalized difference between Negative and Positive classes.



The ranking results are saved in a txt file named "featRank.txt", which is saved in the same folder as the .mat feature files.

13 Status Label

To allow the user to keep track of what is happening in the program, there is a status label on the bottom of the main window. This label gives hints about what the user should do next or shows the current task that the program is working on.



14 Tips

1) Output data and images are saved under a subfolder named \CA_Out\ within the folder where the original image exists, and under filenames indicating the source of the data and the type of output.
2) To read in the output files from CT-FIRE, CT-FIRE output .mat files should be saved in the same folder as the original images.
3) To read in the boundary files (in .csv or .tif format), the boundary files should be saved in the same folder as the original images and should follow the strict naming convention, e.g., if image

is named: "1B-a5.tif", CSV boundary is named "Boundary for 1B-a5.tif.csv", and tiff boundary is named " mask for 1B-a5.tif.tif "

4) Tiff boundary files must be 8bit binary mask images where the inside of a region has a value of 255 and everything else must be 0. Boundary tiff files must have the same number of pixels (length and width) as the original image and may be produced by hand or by segmentation in ImageJ/FIJI.
5) Tiff boundary is recommended for more accurately extracting the features of collagen-cell interaction.
6) -To run feature ranking, an annotation .xlsx file should be prepared and saved in the same folder as the .mat feature files. This file is named as "annotation.xlsx" with two columns, where the first column includes the label for each image(e.g.: 1 for positive, 0: negative), the second column includes the full original image name.

15 Acknowledgement

Guneet Singh Mehta has contributed to this manual.